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Biodegradation of Used Lubricating Oil Containing Hydrocarbon using *Pseudomonas Aeruginosa* and *Rhodococcus Erythopolis*

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ABSTRACT

Engine oil is complex mixture of hydrocarbons and organic compounds used to lubricate parts car engine so the engine works smoothly. After the usage period the oil as a lubricant ends, then the oil will be used more metals and polycyclic aromatic hydrocarbons (PAH). One friendly way environment is by bioremediation, namely biodegradation of pollutant compounds become simpler products and harmless. So far, research on contamination of hydrocarbon compounds especially used oil in fishing ports is still rarely done, especially with the addition of exogenous bacteria. This study aims to analyze the ability of the bacterium Pseudomonas aeuriginosa, Rhodococcus erythropolis and the combination of both in the bioremediation process of used oil hydrocarbons and determine the best bacterial formulations to degrade hydrocarbons from used oil waste. The method used in this research is the experimental method. Significant reduction results occurred in the treatment of combined Pseudomonas aeruginosa and Rhodococcus erythopolis with concentrations of used oil 30 ppm by 70% with the value of used oil concentration of 9 ppm, then in the treatment of Rhodococcus erythopolis with 45 ppm concentration of used oil there was a percentage the lowest decrease of 22% with the value of used oil final concentration of 35 ppm. If referring to the Regulation of the Minister of Environment No. 19 of 2010 that bioremediation of used oil waste using a combination of Pseudomonas aeruginosa and Rhodococcus erythopolis can produce final value pollutants that are still allowed for the harbor's aquatic environment.

Key Words: *Bioremediation, Used Lubricating, Pseudomonas aeruginosa, Rhodococcus erythopolis.*

1. INTRODUCTION

Engine oil is a complex mixture hydrocarbons and organic compounds used to lubricate parts car engine so the engine works smoothly. After usage period the oil as a lubricant ends, then the oil will be used more metals and *polycyclic aromatic hydrocarbons* (PAH). This is often used illegally disposed of in locations that are not should be used to throw away waste, illegal disposal of used oil is an environmental pollution which has global impact and causes Attention that attracts public attention in various countries. PAH are mutagenic and carcinogenic [1]. PAH compound when entering into the blood will be absorbed by the tissue fat and experience oxidation in the liver forming phenol. Next will happen conjugate reaction forms glucoronide which dissolves in water, then goes into kidney. The intermediate compounds formed is epoxide which is toxic and can causing damage to bones marrow. Chronic PAH poisoning can cause abnormalities in the blood, including decreased white blood cells, frozen blood, and red blood cells which causes anemia. As a result, will stimulate the onset of preleukemia, then leukemia which in the end cause cancer [2]. To overcome used oil pollution can be applied to chemical and physical methods, but both of these methods require a fee the high. One friendly way environment is by bioremediation, namely biodegradation of pollutant compounds become simpler products and harmless [3]. Certain types of microbes can only degrade certain hydrocarbon compounds, so that in natural conditions various types of microbes work together (consortium) to degrade contamination of hydrocarbon compounds.

The degradation process of hydrocarbons naturally requires a relatively long time. Therefore, bioremediation technology is widely used to accelerate the process of recovery of waters contaminated with hydrocarbon compounds through regulation of environmental conditions, addition of nutrients (biostimulation) and addition of microbes from outside (bioaugmentation) [9][10]. The application of bioaugmentation techniques is carried out using microbes developed in the laboratory [4]. Some of the advantages offered by this bioaugmentation technique are the time of removal of contaminants in the waters faster and more efficiently compared to the technology of bioremediation without the addition of microbes. Microorganisms which can degrade hydrocarbons petroleum, among others are: *Yokenella* spp., *Alca-ligenes* spp., *Roseomonas* spp., *Stenotropho-monas* spp., *Acinetobacter* spp., *Flavobacter* spp., *Corynebacterium* spp., *Streptococcus* spp., *Providencia* spp., *Sphingobacterium* spp., *Capnocytophaga* spp., *Moraxella* spp., and *Bacillus* spp. [5]. Other organisms such as fungi are also capable degrade hydrocarbons in engine oil to some extent, but requires longer time to grow compared to bacteria [6]. Bioremediation mechanism at the principle is the decomposition process organic material in the biosphere carried out by heterotrophic group of microbes. Heterotrophic microbes have the ability utilize organic compounds, in terms of this is petroleum as a substrate. Decomposition petroleum will produce CO_2, CH_4, water, microbial biomass, and by products in the form of simpler compounds [7]. In general, biodegradation or decomposition of organic compounds by microbes can occur if there is a transformation structure in the compound so that it occurs changes in molecular integrity. Process this is a series of enzymatic chemical reactions which requires appropriate environmental conditions with growth and breeding microbes. So far, research on contamination of hydrocarbon compounds, especially used oil in fishing ports is still rarely done, especially with the addition of exogenous bacteria. This study aims to analyze the ability of the bacterium *Pseudomonas aeuriginosa*, *Rhodococcus erythropolis* and the combination of both in the bioremediation process of used oil hydrocarbons and determine the best bacterial formulations to degrade hydrocarbons from used oil waste.

2. METHODOLOGY

2.1 Materials

The tools used in this study include: 250 ml sample bottles, cool boxes, autoclaves, test tubes, ovens, digital scales, erlenmeyer, magnetic steerers, hot plates, petri dishes, disposable petri, refrigerators, loop needles, ose needle, incubator, bunsen, sprayer, laminary flow, glass object, cover glass, microscope, refrigerator, media container, measuring cup, pipette, test tube rack, haemocytometer, water shaker, vortex mixer, test tube cap, beaker glass, container media scales, pipet volume, separating funnels, spatula, oven, suction balls, film bottles, drop pipet, 1.5 ml microtube, blue tip, yellow tip and white tip. The materials used in this study include: used oil used ships, seawater, bushnell haas agar hydrocarbon selective media, liquid LB (*Luria Bertani*) media, *Rhodococcus erythopolis* isolates, NA (*Nutrient Agar*) media, NaCl solution 0.85%, 3N HCl, n-hexane, Na_2SO_4, Tris-HCl 20 mM buffer pH 7.4, hexadecane solution, DMSO, filter paper, 70% alcohol, cotton, aluminum foil, trypan blue and aquadest.

2.2 Method

The method used in this research is the experimental method. The study was conducted using a complete randomized design arranged in factorial consisting of two factors. Each treatment was carried out with 3 replications.

The first factor is the type of bacterial isolate (K):
K_1: *Pseudomonas aeruginosa*
K_2: *Rhodococcus erythopolis*
K_3: *Pseudomonas aeruginosa* + *Rhodococcus erythopolis*
The second factor is the concentration of used oil (L):
L_1: Concentration of used oil is 15 ppm
L_2: Concentration of used oil is 30 ppm
L_3: Concentration of used oil is 45 ppm

Each of the purified bacterial isolates was taken 1% with a density of 10^8 cell/ml then added to the liquid Bushnell-Haas medium containing 15 ppm, 30 ppm and 45 ppm used oil according to treatment. The culture was incubated at room temperature and shaken on a shaker at a speed of 120 rpm, then an analysis of the density of bacterial cell counts and pH were observed once every 48 hours interval, while the parameters levels of residual used oil (TPH) was observed on days 0, 7 and 14. The same was done for treatment with using both bacteria (mixture of *Pseudomonas aeruginosa* and *Rhodococcus erythopolis*) and for treatment without adding bacteria as a control. The method used in this study was designed based on research conducted and carried out in accordance with the objectives to be achieved. The first step is to isolate the original bacteria from polluted captured degradation of used oil. The second stage is the approval of bacterial inoculants selected from the work of one with the approval given by exogenous bacteria to determine the composition of the bacterial decomposers that are the most optimal in the process of used oil bioremediation.

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3. RESULTS AND DISCUSSION

3.1 Bacterial Density

The growth of microorganisms is an indicator of the process of biodegradation. The growth of microorganisms will increase if they are able to live by utilizing the substrate in the media. To analyze the rate of decrease in hydrocarbons by bacteria, bacterial densities at different times (0, 48, 96, 144, 192, 240, 288 and 336 hours) were observed in this study. The observation of bacterial cell density in each treatment can be seen in Figures 1, 2 and 3.

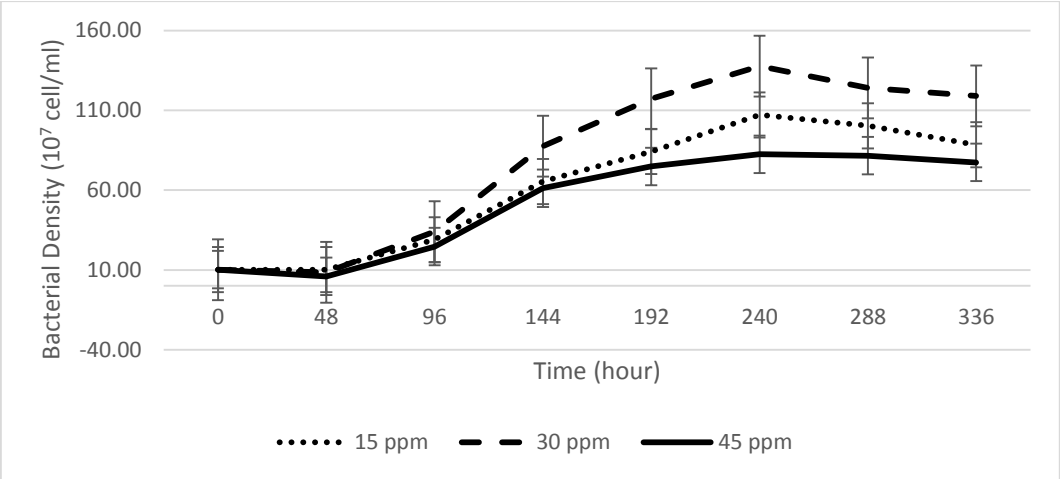

Figure 1. ***Cell Growth Pseudomonas aeruginosa***

Pseudomonas aeruginosa density tends to decrease slightly at the beginning of incubation (0-48 hours). *Pseudomonas aeruginosa* density increased from 96-240 hours with densities ranging from $2.89x10^8$ to $1.38x10^9$. The exponential phase occurs from 48 hours to 240 hours. The optimum phase is reached at 240 hours and at 288 hours *Pseudomonas aeruginosa* experience a decline in population.

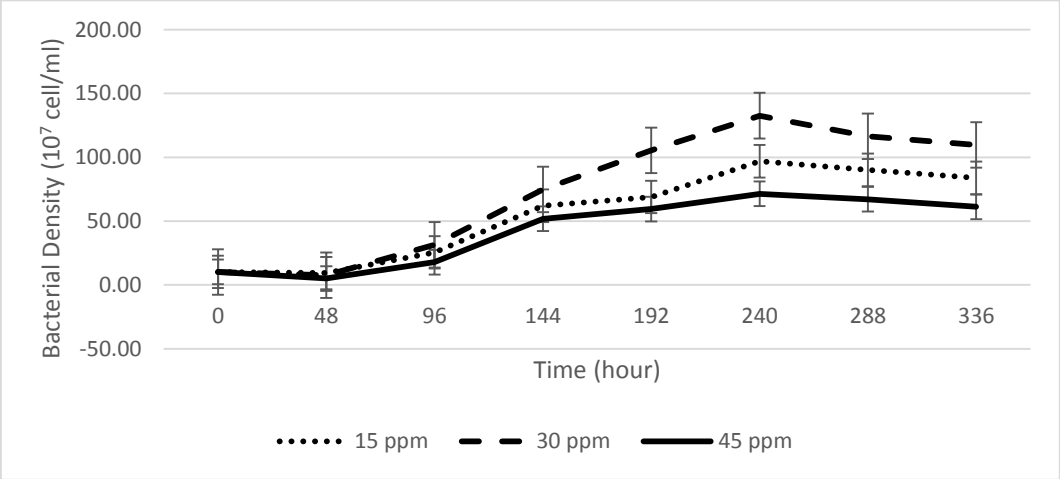

Figure 2. ***Cell Growth Rhodococcus erythopolis***

The density of *Rhodococcus erythopolis* tends to decrease slightly at the beginning of incubation (0-48 hours). The density of *Rhodococcus erythopolis* has increased from 96-240 hours with densities ranging from $1.79x10^8$ to $1.33x10^9$. The exponential phase occurs from the 48 hours to the 240 hours. The optimum phase is reached at 240 hours and at the 288 hours the *Rhodococcus erythopolis* bacteria decrease in population.

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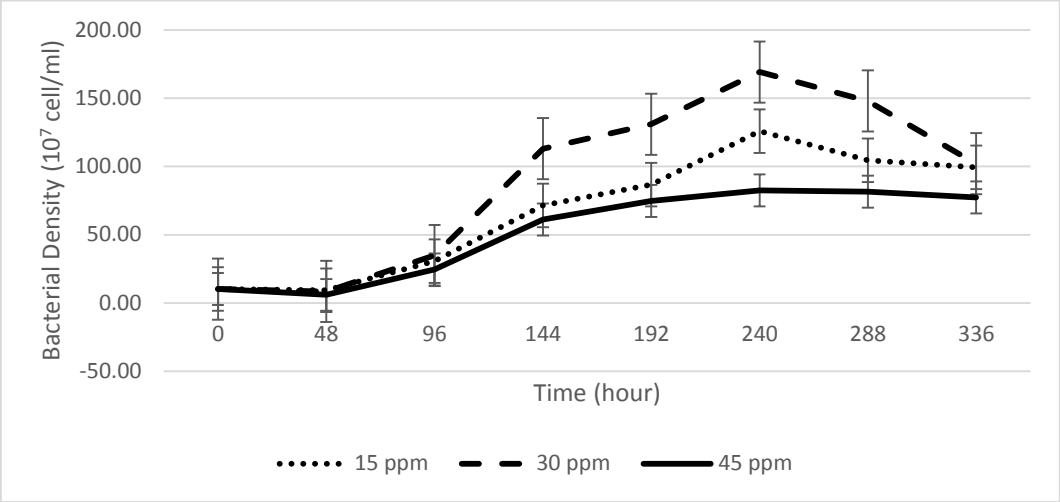

Figure 3. ***Cell Growth P. aeruginosa + R. erythopolis***

The combined cell density between *Pseudomonas aeruginosa + Rhodococcus erythopolis* tended to decrease slightly at the beginning of incubation (0-48 hours). The density of *Pseudomonas aeruginosa + Rhodococcus erythopolis* cells increased from 96-240 hours with densities ranging from $2.47x10^8$ to $1.69x10^9$. The exponential phase occurs from the 48 hours to the 240 hours. The optimum phase is reached at 240 hours and at 288 hours the *Pseudomonas aeruginosa + Rhodococcus erythopolis* cells have a decrease in population. From this density level the combination of *Pseudomonas aeruginosa* and *Rhodococcus erythopolis* produced the highest density of $1.69x10^9$ cell/ml. This was probably due to the bacteria combined with *Pseudomonas aeruginosa* and *Rhodococcus erythropolis* able to grow well on used oil-containing hydrocarbon media. Bioremediation use of indigenous microorganisms (indigen) is still not maximum, so that inoculation of exogenous (exogenous) microorganisms is needed which is a mixed culture of several types of bacteria or fungi that have the potential to degrade these pollutants [8]. The difference in growth in each treatment is due to different adaptation processes. Bacteria will show different growth patterns, periods of time needed to grow and adapt, and metabolites produced [9].

3.2 pH Value

The enzymatic process in bioremediation can cause changes in pH in the media environment so that the pH of the media in this study needs to be observed (Lee *et al*., 2011) (Figures 4, 5 and 6).

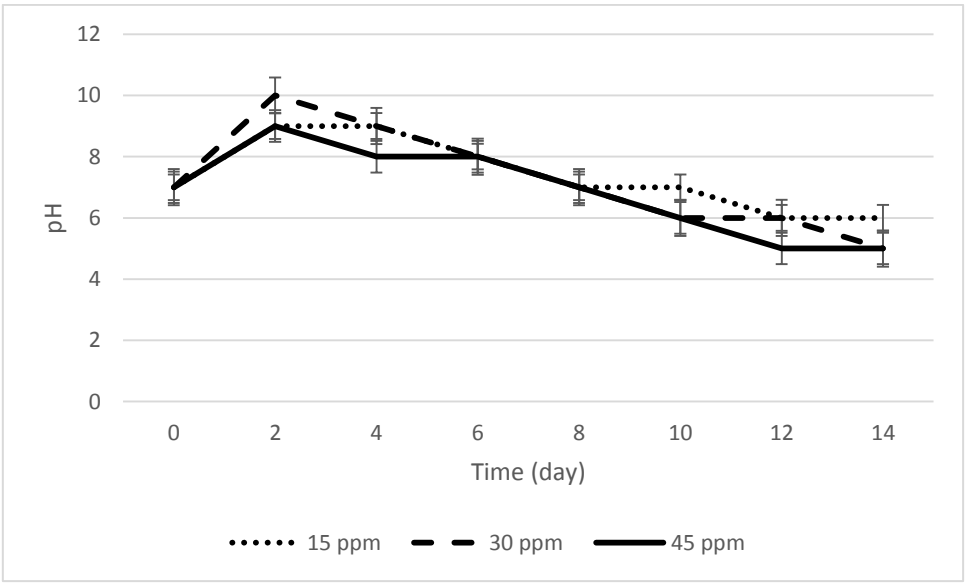

Figure 4. ***pH in Treatment Pseudomonas aeruginosa***

The pH value of *Pseudomonas aeruginosa* culture media tended to increase at the beginning of incubation (0-2 days). The pH value of *Pseudomonas aeruginosa* culture media decreased on day 4 until the end of the study (14 days). Decreasing pH values range from 10 to 5.

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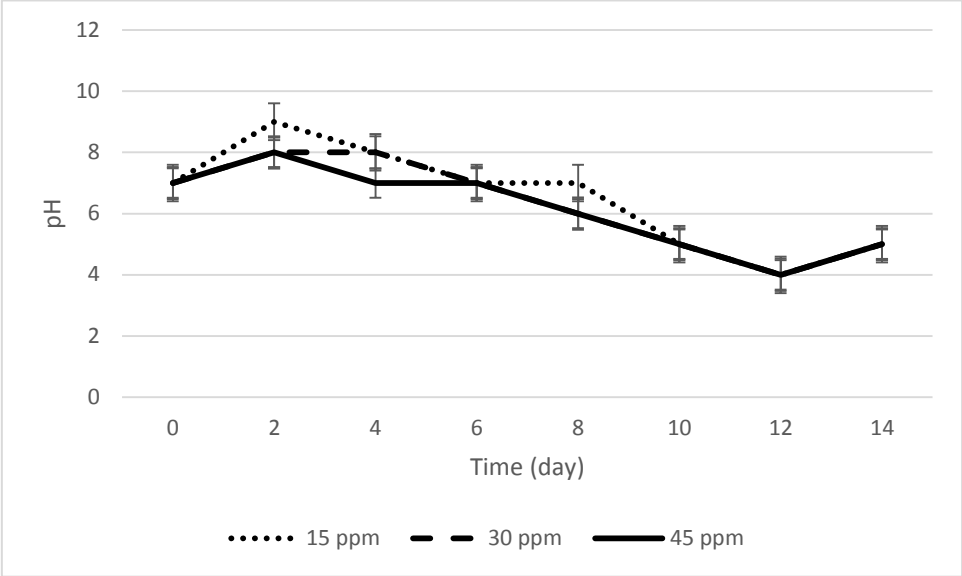

Figure 5. ***pH in Treatment Rhodococcus erythopolis***

The pH value of *Rhodococcus erythopolis* culture media tends to increase at the beginning of incubation (0-2 days). The pH value of *Rhodococcus erythopolis* culture media decreased on day 4 until the end of the study (14 days). The decrease in pH values ranges from 9 to 5.

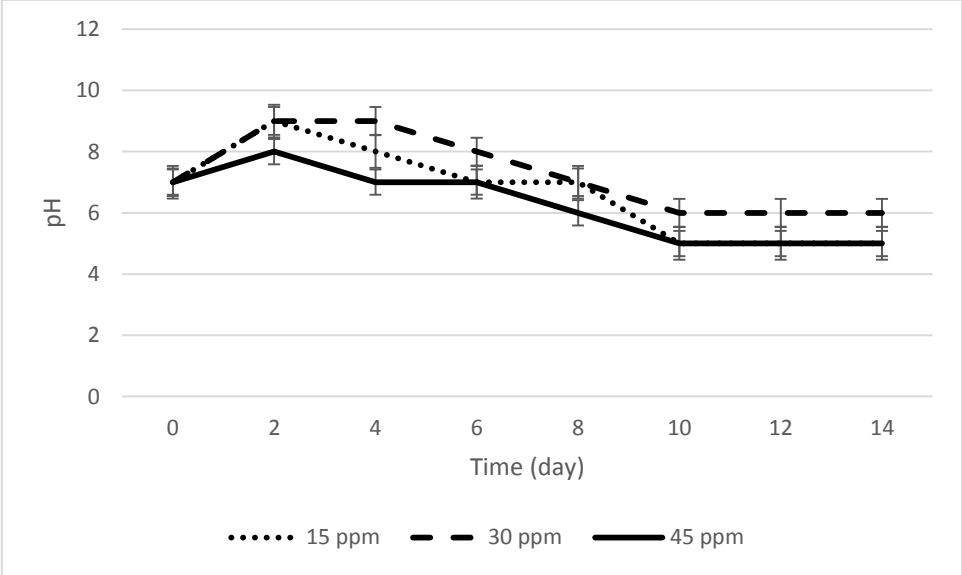

Figure 6. ***pH in Treatment P. aeruginosa + R. erythopolis***

The pH value of the media combining *Pseudomonas aeruginosa* and *Rhodococcus erythopolis* increases the increase at the beginning of incubation (0-2 days). The pH value of the combined culture medium between *Pseudomonas aeruginosa* and *Rhodococcus erythopolis* decreased on the 4 days until the end of the study (14 days). Decreasing pH values up to 9 to 5. Based on the results of the analysis, in general all treatments experienced a decrease in pH value. The decrease in pH value is thought to be caused by the activity of a bacterial consortium that forms acidic metabolites. Biodegradation of alkanes found in used oil will form alcohol and then become fatty acids. The fatty acid from the alkane degradation will be oxidized further to form acetic acid and propionic acid, so that it can reduce the pH value of the medium [11].

3.3 *Total Petroleum Hydrocarbon* (TPH)

TPH measurements were carried out to find out what percentage of the hydrocarbon chain in used oil waste was left after undergoing the bioremediation process. This is done so that dangerous compounds in used oil waste can be minimized so that it is safe for waters. To analyze the effectiveness of *Pseudomonas aeruginosa*, *Rhodococcus erythopolis* and the combination of both in degrading hydrocarbons, 3 concentrations of hydrocarbons from used oil waste were treated in this study (15 ppm, 30 ppm and 45 ppm). The selection of this concentration is based on consideration of the value of hydrocarbon quality standards in the waters.

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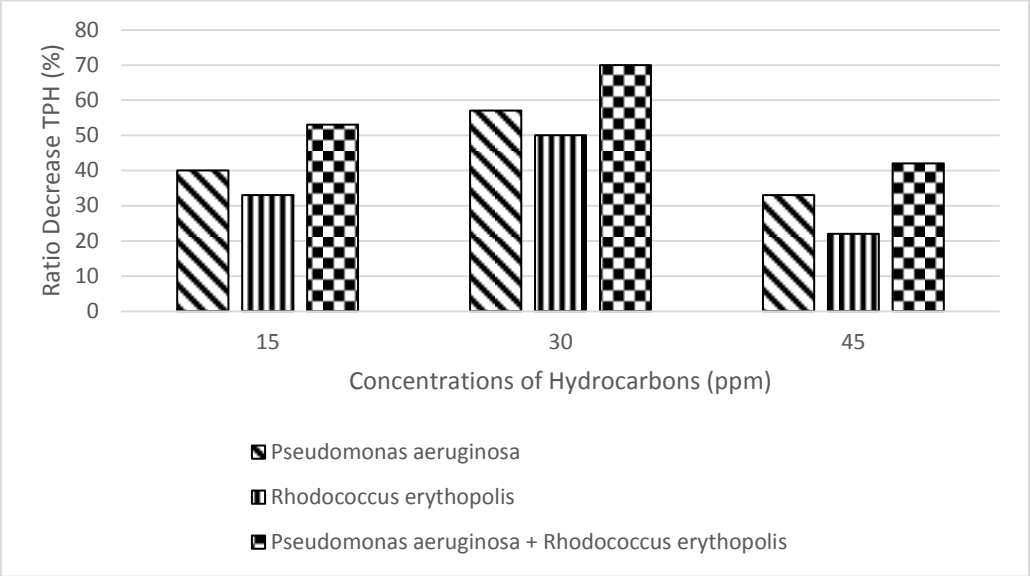

Figure 7. ***Graph of TPH Decrease Percentage***

Based on Figure 7 shows the most significant reduction results occurred in the treatment of combined *Pseudomonas aeruginosa* and *Rhodococcus erythopolis* with concentrations of used oil 30 ppm by 70% with the value of used oil concentration of 9 ppm, then in the treatment of *Rhodococcus erythopolis* with 45 ppm concentration of used oil there was a percentage the lowest decrease of 22% with the value of used oil final concentration of 35 ppm. If referring to the Regulation of the Minister of Environment No. 19 of 2010 concerning waste water quality standards for businesses and/or petroleum processing activities the maximum TPH level is 20 ppm, then bioremediation of used oil waste using a combination of *Pseudomonas aeruginosa* and *Rhodococcus erythopolis* for 14 days at a concentration of used oil waste 30 ppm can produce a final value pollutants that are still allowed for the harbor's aquatic environment. The results of this study indicate a tendency for the greater value of the initial TPH concentration, the greater the decrease in the value of TPH, the largest percentage decrease lies in the TPH concentration of 30 ppm, while the smallest percentage decrease lies in 45 ppm TPH concentration. This is presumably because not too much used oil waste has been added. The less oil waste is added, the smaller the substrate available, so that bacterial activity in the substrate remodel is also low [12].

4. CONCLUSION

Significant reduction results occurred in the treatment of combined *Pseudomonas aeruginosa* and *Rhodococcus erythopolis* with concentrations of used oil 30 ppm by 70% with the value of used oil concentration of 9 ppm, then in the treatment of *Rhodococcus erythopolis* with 45 ppm concentration of used oil there was a percentage the lowest decrease of 22% with the value of used oil final concentration of 35 ppm. If referring to the Regulation of the Minister of Environment No. 19 of 2010 concerning waste water quality standards for businesses and/or petroleum processing activities the maximum TPH level is 20 ppm, then bioremediation of used oil waste using a combination of *Pseudomonas aeruginosa* and *Rhodococcus erythopolis* for 14 days at a concentration of used oil waste 30 ppm can produce a final value pollutants that are still allowed for the harbor's aquatic environment..

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